I hereby certify that I have been publicly appointed and sworn in by the President of the Regional Court (Landgericht) No. I in Munich, Germany, as a translator for the English language. As such I further certify that the following text is a true and complete translation of a Certificate issued by the Swiss Institute for Intellectual Property on January 3, 2000, and of the documents attached to said Certificate. Copies of said documents have been handed over to me.

So declared in Munich,
Federal Republic of Germany,
this 5th day of November 2003

Helga Rebstock
(publicly commissioned
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The enclosed papers are in conformity with the original technical documents of the patent request for Switzerland and Liechtenstein, as specified on the next page. Switzerland and the Principality of Liechtenstein form a unitary territory of protection. Therefore, protection can only be requested for both countries together.

Bern, January 3, 2000

Swiss Institute for Intellectual Property

Patent Administration

(signature)
Rolf Hofstetter

Patent Request No. 1999 0279/99

CERTIFICATE OF DEPOSIT (Art. 46 para. 5 Ordinance Concerning Patents)

The Swiss Institute for Intellectual Property certifies receipt of the Swiss patent request specified in more detail below.

Title:
Heat shock-induced promoter.

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Probable classes: C12N

Heat shock-induced promoter

The present invention relates to a heat shock-induced promoter, a method for producing the same, and to the use thereof. In particular, the present invention relates to a selectively heat shock-induced promoter, specifically a heat shock-induced promoter which is also active at high temperatures and preferably in fungi, specifically preferably in yeasts.

There are numerous industrial applications where it is highly desirable that microorganisms propagate under conditions which are not normal, but are also not lethal yet, and that they express a desired target product under such conditions.

Microorganisms are able to respond to a very great number of stress situations (Winderickx et al., 1996), such as heat or cold shock, ethanol, heavy-metal ions, oxygen deprivation, or nutrient deprivation, in particular glucose deprivation.

Yeasts and other fungi are known to accumulate trehalose during phases of reduced growth (Küenzi & Fichter, 1972). These are most of the time those stages of development that, for example, are tolerant of water deprivation and heat, such as spores, conidiae, sclerotia, or cells in the stationary growth phase. It is also already known that *Saccharomyces cerevisiae* cells accumulate trehalose during a one-hour heat shock from 27°C to 40°C and that the trehalose accumulation correlates with an increased thermotolerance (Hottiger et al., 1987). Selective mutations have been used to demonstrate that trehalose is indeed a necessary factor for the induction of thermotolerance (De Virgilio et al., 1994).

HSEs (heat shock elements) and STREs (stress responsive elements) are present in the promoter regions of stress-induced genes, such as the genes of *S. cerevisiae* responsible for the trehalose synthesis. These elements appear to mediate activation of stress genes by stress induction, including heat shock induction. It is now generally

accepted that phosphorylation of Msn2p and Msn4p via the Ras/cAMP pathway inhibits the Msn2p and Msn4p transcription factors. In the absence of this inhibition (e.g. under stress conditions) Msn2p and Msn4p become active. STREs with the sequence CCCCT (Kobayashi and McEntee, 1993) are attributed with a role in the response to the stress conditions.

Yeast organisms are very well suited for the commercial expression of heterologous proteins. Often they have advantages over tissue cultures and bacteria. Conventional yeasts, however, have the drawback that they show an optimum growth at about 30°C and already experience 40°C as a heat shock. Due to their low temperature for optimum growth they are very prone to contamination by other microorganisms. It is therefore highly desirable to employ a yeast culture which can be cultivated at a considerably higher temperature so as to keep contamination with other microorganisms at a minimum level.

A very heat-tolerant yeast cell is *Hansenula polymorpha*. *H. polymorpha* belongs to a small group of so-called methylotrophic yeasts which can exploit methanol as a carbon and energy source. *H. polymorpha* was isolated by incubation at 37°C from soil samples (Leven & Cuoni, 1973)

Apart from a reduced risk of contamination, a yeast culture which also exhibits good growth characteristics at a high temperature has the advantage that large fermenting cultures which warm up rapidly must be cooled less strongly, which has an advantageous effect on the costs.

The promoters employed nowadays for heat shock induction in yeasts have the drawback that they do not respond selectively to heat shock. Their mechanism of activation and deactivation can therefore not be controlled sufficiently well, which can cause problems in particular during the production of proteins which are damaging to cells. The *TPS1* promoter from *S. cerevisiae*, for example, exhibits several sequences known to be general stress elements (STRE elements), namely CCCCT and AGGGG,

but not more than one sequence acting as a heat shock element, namely GGAACAGAACAATCG. In addition, owing to their wide stress response, the promoters currently known are activated by only one stress factor to a degree which is not satisfactory for many applications.

An additional problem consists in the expression of heterologous genes in *H. polymorpha*, i.e. promoters which are suited for a cell cultivated at such high temperatures have so far not been known to permit an expression in a selective way only (or at least preferably) under conditions experienced by said cell as stress, so that a synthesis of proteins which are critical for the survival of *H. polymorpha* has so far not been possible or has only been possible to a limited degree.

It has therefore been the object of the present invention to provide a promoter the heat shock-induced characteristic of which is as selective as possible, specifically a promoter which is active in yeasts, and which is also suitable for protein expression at high temperatures.

Surprisingly, the promoter of the trehalose-6-phosphate synthase (*TSP1*) gene of *Hansenula polymorpha* has now been found to contain, at least in the first 300 bp upstream of the coding sequence, none of the STRE elements which were found in *S. cerevisiae* and which were assumed to be primarily responsible for the stress response including heat-shock induction of this gene. This promoter was further found to respond well and very selectively to heat.

A subject matter of the present application is therefore a promoter which is characterized in that it contains or consists of a partial sequence which over a length of 300 bp exhibits at least 40% identity with the following sequence:

```
-792  CTTAAATACCACAATAGGAAAATTATCAATAAAGCTTTTCGGATTTCATTACGTTATATC -733
-732  GCAAAAAAATAGTCGAGCTTTCTGAACCGTTCGTTAATAAAAAAATAGTTTTTTTCAGATT -673
-672  TCTATGTGAGGCAGTCACGATAGAATTCCATCGAACTCGTCAGCGCCAAATGTGAATGCG -613
-612  GCTTTCAAAAGCTTTGTCGAATTTGGGATGGGAATCCATGAATCGAAGATGTCAAAATGG -553
-552  GGGATCACAAAAGTACACTCACGAGGAAAATCAAAACCTTCTCGTACCTTTAACACATAC -493
-492  GGAAATGATCGATCGATTTGAGAAGATTCCTCAATGATTTTCGTCATATATAGGTATCTG -433
-432  AGGTATTTATGGACCGATTCGTAATAACATCATATACATCGCGCTTTGTCCCTGTCCCAG -373
-372  AGATTTCGATGAAAAAAGCGAATTTTATTCTAATATTTGAAGCATGCCAAACATGGGGCA -313
-312  GTTGATTTGTGTGAGGGTAAAATATCATGAATTGCACCCATCAAATGCAGCAAGATATTG -253
-252  ACCAATCCTATAATAGAAAACAGACTTACCACAAATAGATTGTGATGACGATATTATGAA -193
-192  TCTCCAGATGAAAGGCTCGAAAGCTATGAAGCCTCTTGAAACTTTTCATGGTGAGATAAT -133
-132  ATTTTCGAAATTTCCACGAACTTCTAAAACGCAATTATTGAATATAAAGGAAAAATAATA -73
 -72  TTTCCATATAGCAAGCAAATCAAGCTGCACTCCTCATCCTTAAAACTAATAAATCTTACC -13
 -12  CATTTGATACCA
```

Further subject matters of the present invention include a method for isolating a promoter according to the invention, as well as the use of such a promoter.

The invention is now described in closer detail with reference to the figures, which show the following:

Figure 1 shows growth curves of *H. polymorpha* at 27°C, 37°C and 47°C.

Figure 2 shows the vitality following entry into the stationary phase at 27°C, 37°C and 47°C.

Figure 3 A shows a Northern blot of the heat shock.

Figure 3 B shows the correlation of the increase in *TPS1* mRNA with the increase in Tps1 protein (Tps1p).

Figure 3 C shows the correlation between the increase in *TPS1* mRNA and the increase in trehalose-6-phosphate synthase activity and the increase in the intracellular trehalose concentration.

Figure 4 A shows the correlation of the trehalose accumulation with the increase in trehalose-6-phosphate synthase activity during glucose deprivation.

Figure 4 B shows the correlation of the trehalose accumulation with the increase in *TPS1* mRNA during glucose deprivation.

Figure 4 C shows the correlation of the trehalose accumulation with the increase in Tps1 protein during glucose deprivation.

Fig. 5 shows the homology of certain DNA sequence regions of trehalose-6-phosphate synthase from a number of organisms, on the basis of which the original isolation of the *TSP1* gene of *H. polymorpha* was carried out.

As has already been mentioned above, the present invention relates to a heat shock-induced promoter which contains or consists of a partial sequence which over a length of 300 bp exhibits at least 40% identity with the following sequence or the complementary sequence thereof:

```
 -792  CTTAAATACCACAATAGGAAAATTATCAATAAAGCTTTTCGGATTTCATTACGTTATATC  -733
 -732  GCAAAAAAATAGTCGAGCTTTCTGAACCGTTCGTTAATAAAAAAATAGTTTTTTCAGATT  -673
 -672  TCTATGTGAGGCAGTCACGATAGAATTCCATCGAACTCGTCAGCGCCAAATGTGAATGCG  -613
 -612  GCTTTCAAAAGCTTTGTCGAATTTGGGATGGGAATCCATGAATCGAAGATGTCAAAATGG  -553
 -552  GGGATCACAAAAGTACACTCACGAGGAAAATCAAAACCTTCTCGTACCTTTAACACATAC  -493
 -492  GGAAATGATCGATCGATTTGAGAAGATTCCTCAATGATTTTCGTCATATATAGGTATCTG  -433
 -432  AGGTATTTATGGACCGATTCGTAATAACATCATATACATCGCGCTTTGTCCCTGTCCCAG  -373
 -372  AGATTTCGATGAAAAAAGCGAATTTTATTCTAATATTTGAAGCATGCCAAACATGGGGCA  -313
 -312  GTTGATTTGTGTGAGGGTAAAATATCATGAATTGCACCCATCAAATGCAGCAAGATATTG  -253
 -252  ACCAATCCTATAATAGAAAACAGACTTACCACAAATAGATTGTGATGACGATATTATGAA  -193
 -192  TCTCCAGATGAAAGGCTCGAAAGCTATGAAGCCTCTTGAAACTTTTCATGGTGAGATAAT  -133
 -132  ATTTTCGAAATTTCCACGAACTTCTAAAACGCAATTATTGAATATAAAGGAAAAATAATA  -73
  -72  TTTCCATATAGCAAGCAAATCAAGCTGCACTCCTCATCCTTAAAACTAATAAATCTTACC  -13
  -12  CATTTGATACCA
```

Within the scope of the following further description, the indicated sequences always include the complementary sequences thereof.

Preferably, the promoter according to the invention does not contain STRE elements with the sequence CCCCT or AGGGG within the partial sequence.

Preferred promoters according to this invention contain, within the partial sequence, at least one heat shock element having the sequence NGAANNXYZNNGAAN or the complementary sequence thereof, where N, X, Y and Z independently of one another may be A, T, C and G, where X is preferably G, C or T, Y preferably A or T, and Z preferably C or A. Specifically preferred are heat shock elements selected from the sequences TGAAGCCTCTTGAAA and/or TGAATATAAAGGAAA and/or the complementary sequences thereof. There may be the same or different HSEs,

preference being given to different HSEs and to the presence of at least two of such elements.

A particularly preferred promoter comprises the sequence of nucleotide –166 to nucleotide –80 (see also Table 1 in which two HSEs are marked by being underlined), preferably a sequence directly following the coding sequence and having the above-indicated length of at least 300 bp and the minimum identity of 40%. A sequence which is much preferred at the moment corresponds to the above-indicated sequence and thus contains two different HSEs, namely TGAAGCCTCTTGAAA and TGAATATAAAGGAAA.

Table 1:

```
-792  CTTAAATACCACAATAGGAAAATTATCAATAAAGCTTTTCGGATTTCATTACGTTATATC -733
-732  GCAAAAAAATAGTCGAGCTTTCTGAACCGTTCGTTAATAAAAAAATAGTTTTTTCAGATT -673
-672  TCTATGTGAGGCAGTCACGATAGAATTCCATCGAACTCGTCAGCGCCAAATGTGAATGCG -613
-612  GCTTTCAAAAGCTTTGTCGAATTTGGGATGGGAATCCATGAATCGAAGATGTCAAAATGG -553
-552  GGGATCACAAAAGTACACTCACGAGGAAAATCAAAACCTTCTCGTACCTTTAACACATAC -493
-492  GGAAATGATCGATCGATTTGAGAAGATTCCTCAATGATTTTCGTCATATATAGGTATCTG -433
-432  AGGTATTTATGGACCGATTCGTAATAACATCATATACATCGCGCTTTGTCCCTGTCCCAG -373
-372  AGATTTCGATGAAAAAAGCGAATTTTATTCTAATATTTGAAGCATGCCAAACATGGGGCA -313
-312  GTTGATTTGTGTGAGGGTAAAATATCATGAATTGCACCCATCAAATGCAGCAAGATATTG -253
-252  ACCAATCCTATAATAGAAAACAGACTTACCACAAATAGATTGTGATGACGATATTATGAA -193
-192  TCTCCAGATGAAAGGCTCGAAAGCTATGAAGCCTCTTGAAACTTTTCATGGTGAGATAAT -133
-132  ATTTTCGAAATTTCCACGAACTTCTAAAACGCAATTATTGAATATAAAGGAAAAATAATA -73
 -72  TTTCCATATAGCAAGCAAATCAAGCTGCACTCCTCATCCTTAAAACTAATAAATCTTACC -13
 -12  CATTTGATACCAATGGTCAAAGGTAATGTTATAGTGGTTTCAAATAGAATCCCAGTCACT  48
   1                MetValLysGlyAsnValIleValValSerAsnArgIleProValThr  16
  49  ATTAAGAAGACTGAAGATGATGAAAATGGAAAATCAAGATACGACTATACAATGTCATCA 108
  17  IleLysLysThrGluAspAspGluAsnGlyLysSerArgTyrAspTyrThrMetSerSer  36
 109  GGCGGATTAGTGACGGCATTACAAGGGCTCAAAAATCCATTTCGATGGTTTGGATGGCCT 168
  37  GlyGlyLeuValThrAlaLeuGlnGlyLeuLysAsnProPheArgTrpPheGlyTrpPro  56
 169  GGGATGTCTGTTGATAGCGAACAGGGACGACAAACTGTCGAGCGGGATTTGAAGGAAAAG 228
  57  GlyMetSerValAspSerGluGlnGlyArgGlnThrValGluArgAspLeuLysGluLys  76
 229  TTCAATTGTTATCCGATATGGTTAAGTGACGAAATTGCAGACTTACATTATAACGGCTTT 288
  77  PheAsnCysTyrProIleTrpLeuSerAspGluIleAlaAspLeuHisTyrAsnGlyPhe  96
 289  AGCAATTCTATACTTTGGCCATTGTTCCACTATCACCCAGGGGAGATGAATTTTGATGAA 348
  97  SerAsnSerIleLeuTrpProLeuPheHisTyrHisProGlyGluMetAsnPheAspGlu 116
 349  ATTGCTTGGGCCGCTTATTTGGAAGCAAATAAACTGTTTTGCCAAACGATCTTAAAGGAG 408
 117  IleAlaTrpAlaAlaTyrLeuGluAlaAsnLysLeuPheCysGlnThrIleLeuLysGlu 136
 409  ATAAAAGACGGGGACGTTATCTGGGTACATGATTATCATCTCATGTTGTTGCCTTCACTG 468
 137  IleLysAspGlyAspValIleTrpValHisAspTyrHisLeuMetLeuLeuProSerLeu 156
 469  CTAAGAGACCAACTTAATAGTAAGGGGCTACCGAATGTCAAAATTGGCTTTTTCCTTCAT 528
 157  LeuArgAspGlnLeuAsnSerLysGlyLeuProAsnValLysIleGlyPhePheLeuHis 176
 529  ACTCCTTTTCCTTCAAGCGAAATATACAGGATACTTCCTGTAAGGAAAGAAATTCTCGAA 588
 177  ThrProPheProSerSerGluIleTyrArgIleLeuProValArgLysGluIleLeuGlu 196
 589  GGAGTGCTTAGTTGTGATTTGATAGGTTTCCACACCTATGATTATGTCCGTCACTTTCTT 648
 197  GlyValLeuSerCysAspLeuIleGlyPheHisThrTyrAspTyrValArgHisPheLeu 216
 649  AGTTCGGTTGAAAGAATATTGAAATTGCGAACGAGCCCACAAGGTGTTGTCTATAATGAT 708
 217  SerSerValGluArgIleLeuLysLeuArgThrSerProGlnGlyValValTyrAsnAsp 236
 709  AGACAGGTGACTGTAAGTGCTTATCCGATTGGCATTGACGTTGACAAATTCTTGAATGGT 768
 237  ArgGlnValThrValSerAlaTyrProIleGlyIleAspValAspLysPheLeuAsnGly 256
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       769  CTTAAGACTGATGAGGTCAAAAGCAGGATAAAACAGCTGGAAACCAGATTTGGTAAAGAT  828
       257  LeuLysThrAspGluValLysSerArgIleLysGlnLeuGluThrArgPheGlyLysAsp  276
       829  TGTAAACTTATTATTGGGGTGGACAGGCTGGATTACATCAAAGGTGTACCTCAAAAACTC  888
       277  CysLysLeuIleIleGlyValAspArgLeuAspTyrIleLysGlyValProGlnLysLeu  296
  5    889  CACGCGTTTGAAATTTTCTTGGAGAGACACCCTGAGTGGATTGGAAAAGTTGTTTTGATA  948
       297  HisAlaPheGluIlePheLeuGluArgHisProGluTrpIleGlyLysValValLeuIle  316
       949  CAGGTGGCTGTCCCCTCACGAGGGGACGTTGAAGAATATCAATCTTTGAGGGCAGCTGTA  1008
       317  GlnValAlaValProSerArgGlyAspValGluGluTyrGlnSerLeuArgAlaAlaVal  336
      1009  AATGAGCTAGTGGGAAGAATCAATGGTAGATTTGGTACCGTCGAATTTGTTCCTATCCAT  1068
 10    337  AsnGluLeuValGlyArgIleAsnGlyArgPheGlyThrValGluPheValProIleHis  356
      1069  TTCCTTCATAAAAGCGTGAACTTCCAAGAGCTGATATCTGTCTACGCTGCTAGTGATGTT  1128
       357  PheLeuHisLysSerValAsnPheGlnGluLeuIleSerValTyrAlaAlaSerAspVal  376
      1129  TGTGTAGTGTCATCGACACGGGACGGAATGAATTTGGTCAGTTATGAATACATTGCTTGT  1188
       377  CysValValSerSerThrArgAspGlyMetAsnLeuValSerTyrGluTyrIleAlaCys  396
 15   1189  CAACAAGATCGAAAGGGATCTCTAGTACTAAGTGAATTTGCGGGAGCTGCTCAGTCATTA  1248
       397  GlnGlnAspArgLysGlySerLeuValLeuSerGluPheAlaGlyAlaAlaGlnSerLeu  416
      1249  AATGGCGCTCTCGTAGTGAATCCATGGAATACAGAAGAACTCAGTGAAGCTATTTACGAA  1308
       417  AsnGlyAlaLeuValValAsnProTrpAsnThrGluGluLeuSerGluAlaIleTyrGlu  436
      1309  GGCTTGATCATGAGTGAAGAGAAAAGGAGGGGCAATTTTCAGAAGATGTTCAAGTACATT  1368
 20    437  GlyLeuIleMetSerGluGluLysArgArgGlyAsnPheGlnLysMetPheLysTyrIle  456
      1369  GAGAAATATACTGCAAGTTATTGGGGAGAGAACTTTGTGAAAGAATTGACGAGAGTGTGA  1428
       457  GluLysTyrThrAlaSerTyrTrpGlyGluAsnPheValLysGluLeuThrArgValEnd  476
      1429  TTACTGTGGTTTGCAGGTTAATTTGAAATGTTCACTTGTACTTGAAGAATTTTATATTAT  1488
      1489  ATACATGTTATACATCAATAGGATAAAAATTAAGTAGACAAAGTTATCATTTTGTTGGGC  1548
 25   1549  TGTAAAAATTGAACGATAACAATATATTTGACAAAATTAATTTGATCTAATTGAGCTGGA  1608
      1609  GGGCGTAATATATTTGGTTTCCTGAATCATCTTGTAGATCACAATATGGGGCAGCTTCTT  1668
      1669  TCGCAGCCGATCACAGAGAAACACATCACACTTGTCCAACATGATCACATATCGCATTCA  1728
      1729  ATCGGGGAAATGCAAGGATACAGGTTGACCATGGAAGACGCGTTCTGTGATTTGAACGAA  1788
      1789  AGAATATTCGTGACGGAAGAGGGACTTGACATCAGAAAACAAGACGAGAATACAGAGGGT  1848
 30   1849  GATCTGGAGTCTCTTCAAATTAACATTTATGGTGTCTTTGACGGACATGGCGGTT       1903
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A preferred promoter of the present invention is a promoter obtainable by isolating the *TPS1* gene from a suitable gene library, and parts of such a promoter as described above. Such promoters include effective alleles and mutants of the above-described promoter sequence as shown in Table 1.

Preferred partial sequences which comprise at least two HSEs may be present within a promoter according to the invention either once or several times.

Such promoters may either be prepared synthetically by conventional methods, or isolated from suitable DNA libraries and subsequently mutated as required. The preparation of such libraries is also known to a person skilled in the art. Isolation is preferably performed by preparing a probe with a length of at least 200 - 400 bp and the coding sequence of the *TPS1* (see Table 1), which is used to screen a DNA library, in

particular a genomic DNA library. A probe of this kind can be prepared by means of PCR (polymerase chain reaction) using suitable primers, each of which should preferably be at least 20 – 21 bp in length and possess suitable sequences according to Table 1 (or the corresponding complementary sequence), and genomic DNA or cDNA from *H. polymorpha* as a "template".

Probes may either be synthesized, or prepared by fragmentation of available TPS1 DNA where applicable.

It is of course also possible to screen directly by means of probes that correspond to parts of the promoter sequence; this procedure is less preferable, however, owing to the at best incomplete conservation of the sequence within non-coding parts.

The promoters of the present invention are very well suited for use in the heat shock-induced expression of exogenous DNA in eukaryotes, specifically fungi and in particular yeasts.

Owing to heat shock induction the expression of a foreign gene can largely be suppressed in certain growth stages, and damage to the cell or cell mass can thus be reduced considerably.

Owing to their ability to perform cotranslational and postranslational modifications which are similar to the human modifications, yeasts are preferred organisms.

A particularly preferred yeast for foreign gene expression is *Hansenula polymorpha* because of its temperature resistance. This cell is particularly preferred for the use of a promoter isolated from the cell itself because it contains all factors needed for achieving the astonishingly high expression.

The foreign genes suited for the expression under the control of the promoter of the invention are not specifically limited, but particularly suited are those genes the

expression product of which must e.g. be glycosilated, and which can thus not be produced in bacterial cultures.

The high temperature at which *H. polymorpha* continues to grow and produce protein enables other undesired organisms to be eliminated. The reason for this is that *H. polymorpha* has been shown not only to possess a very high optimum growth temperature, in the region of 37°C, but also to be able to survive temperatures of approximately 50°C unharmed (see Figure 1). The vitality of *H. polymorpha* following entry into the stationary phase does not fall for some 50 hours even at 47°C (Figure 2).

A promoter isolated from *H. polymorpha* itself and its mode of action are described in greater detail below. This promoter, which controls the expression of *TPS1*, was studied by measurement of the increase in *TPS1* mRNA under certain conditions. It was found that whilst this promoter expressed small quantities of *TPS1* at temperatures very low for *H. polymorpha*, the expression increased very strongly at high temperatures, i.e. much more strongly than is the case with heat shock-induced promoters previously described (see Figure 3A, Northern blot of the heat shock). The heat-induced increase in *TPS1* mRNA correlates with the increase in Tps1 protein (Figure 3 B), with the increase in trehalose-6-phosphate synthase activity, and with the increase in the intracellular trehalose concentration (Figure 3 C). In order to optimize the thermal influence, the promoter can for example be selectively shortened and coupled with further segments containing HSE.

In addition to heat induction, a trehalose accumulation dependent upon the glucose deprivation was also observed, as anticipated owing to the close biological relationship between these two stress factors (see Figure 4 A). This trehalose accumulation correlates with the increase in trehalose-6-phosphate synthase activity, the increase in *TPS1* mRNA (Figure 4 B), and the increase in trehalose accumulation observed with the increase in Tps1 protein during glucose deprivation (Figure 4C).

The extremely high accumulation of *TPS1* mRNA indicates that the *TPS1* mRNA is highly stable, which makes it (and the cDNA based upon it or information obtainable from it) not only a valuable tool for isolation of the promoter, but also a particularly valuable means for protecting other organisms against a range of stress conditions, such as heat or drought. *TPS1* DNA provided with suitable promoters and vectors (for example as described in WO 93/17093 and WO 96/00789) can for example be employed to protect plants against water deprivation, thus enabling them to be cultivated in warmer regions and regions with lower precipitation. Not only *TPS1* DNA, but also DNA related to it can of course also be employed for this purpose. Such DNA comprises DNA sequences coding for amino acid sequences, exhibiting at least 80% identity with the sequence of *TPS1* of *Hansenula polymorpha* as shown in Table 1, preferably 90%, particularly preferably 100% identity. The DNA sequences of the present invention also relate to amino acid sequences which comprise at least a partial sequence of the above-defined sequences and exhibit trehalose-6-phosphate synthase activity, furthermore DNA sequences which are characterized in that they exhibit at least 80%, preferably 90%, particularly preferably 100%, identity or complementarity with the sequence shown in Table 1 or with a sequence which in consideration of the degeneration of the genetic code codes for a corresponding amino acid sequence, or that it hybridizes under stringent conditions with the sequence shown in Table 1 or a sequence which in consideration of the degeneration of the genetic code codes for a corresponding amino acid sequence.

The present invention further relates to corresponding amino acid sequences. Specifically preferred amino and nucleic acid sequences are, apart from the sequences shown in Table 1, alleles thereof.

The invention is now described in more detail with reference to examples. These examples serve to illustrate the invention and should not be interpreted as limiting the scope of the invention in some way or other.

Examples

General information on materials and methods:

Special reagents and materials

Bio 101, Vista, USA	Geneclean II Kit
gBioRad Lab., Munich, Germany	BioRad Protein Assay (Bradford)
Boehringer, Mannheim, Germany	GOD/POD kit for glucose measurement, ethanol kit, "COMPLETE" proteinase inhibitor cocktail tablets
Fluka Chemie AG, Buchs, Switzerland	Cycloheximide (Actidion), SDS, D+trehalose, PEP, TRICIN, NADH, Folin-Ciocalteu phenol reagent
ICN Biochemicals, Ohio, USA	"Liquigel" 40% acrylamide/N'N'-methylene-bisacrylamide (37.5:1)
Kodak, New York, USA	BIOMAX MR scientific imaging film
Mediatech, Herndon, USA	Geneticin G418 sulphate (antibiotic)
Perkin Elmer Applied Biosystems, Forest City, USA	DNA sequencing kit
Pharmacia Biotech, Sweden	Nap-10 columns (with Sephadex G-25), all restriction enzymes used, Taq polymerase
Qiagen GmbH, Germany	Plasmide Midi Kit (50)
Schleicher + Schuell, Dassel, Germany	Protran BA 83 0.2 µm/ ∅ 82 mm (cellulose nitrate round filter), Protran BA 83 0.2 µm (transfer membrane for blots)
Sigma, St. Louis, USA	Monoclonal goat anti-rabbit immunoglobulins (alkaline phosphatase conjugate), trehalase from pig kidneys (Cat. No. T-8778), UDPG, G6P, LDH, pyruvate kinase

Stratagene, La Jolla, USA	Prime-It II kit (random primer labelling kit), NucTrap columns (probe purification columns incl. push column beta shield device)
US Biological, Swampscott, USA	Bacteriological Agar, YPD broth enhanced formulation W/Peptone X, LB broth Miller

Apparatus used

Electroporation unit	*E. coli* pulser, BioRad Laboratories, Hercules USA
HPLC	DIONEX DX-300, DIONEX, Sunnyvale, USA
Cooling centrifuges	Centrikon H-401, Kontron Instr. AG, Zürich, Switzerland
	IEC Centra GP8R, Brouwer, Lucerne, Switzerland
	Biofuge 17RS, Heraeus Sepatech, Germany
PCR apparatus	Progene, Techne, Cambridge, United Kingdom
Phosphoimager	GS 250 Molecular Imager (including associated equipment), BioRad Laboratories, Hercules, USA
Photometer	Anthos 2001 (for microtiter plates), Anthos Labtec Instruments, Salzburg, Austria
	Shimadzu UV-160A, Japan
Sequencer	ABI PRISM 301 Genetic Analyzer, Perkin Elmer, Applied Biosystems, Foster City, USA

Bacterial strain and culture conditions

All transformations in connection with cloning of the TPS1 gene of *H. polymorpha* were carried out in the *E. coli* strain DH5α (Gibco BRL), the standard protocols (Sambrook *et.*

al., 1989) being followed. The medium for *E. coli* was also produced in accordance with a standard recipe (Sambrook *et al.*, 1989).

Isolation of plasmid DNA from *E. coli* (STET prep)

Plasmid DNA was isolated in accordance with a modified protocol according to Sambrook *et al.* (1989). A spatula was used to scrape cell material from a plate. This material was then added to 500 µl STET (8% [w/v] sucrose, 5% [v/v] Triton X-100, 50 mM EDTA, 50 mM Tris-HCl, pH 8.0) with 35 µl lysozyme (10 mg/ml) and mixed. The samples were then boiled for 1 min 40 s at 100°C and centrifuged for 10 minutes at 20,000 g and 4°C. Approx. 400 µl of supernatant was drawn by means of a pipette, and the DNA precipitated using 400 µl isopropanol. Following centrifugation for 10 minutes at 20,000 g and 4°C, the entire supernatant was discarded and the DNA pellet washed once with ice-cold 70% [v/v] ethanol. Finally, the DNA was dried at room temperature and suspended in 50-70 µl TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0).

Yeast strain and culture conditions

The yeast strain employed in this work was a wild-type *Hansenula polymorpha* (No. 196 I/9 of the yeast collection), obtained from P. Piper, London (1994). Stock cultures were grown on YPD Agar (2% [w/v] glucose, 2% [w/v] bactopeptone, 1% [w/v] yeast extract, 2% [w/v] agar) and re-stocked every six weeks. They served as inoculum for YPD liquid cultures (composition the same as YPD agar, but without 2% [w/v] agar).

For the cultivation of cell cultures, autoclaved liquid media were inoculated with stock culture and incubated overnight in shaking incubators at 27°C, 37°C or 47°C, depending upon the experiment.

Determination of the optical density of the *H. polymorpha* cell cultures

In order to determine the optical density (OD), 200 µl (suitably diluted where applicable with YPD) cell culture was placed in a vial of a microtitre plate and measured at 620 nm using an Anthos 2001 photospectrometer. 200 µl YPD was employed as the blank.

Growth and heat shock experiments with *H. polymorpha*

Overnight cultures were used to inoculate YPD medium in Erlenmeyer flasks. Care was taken to inoculate this preculture at the temperature at which the experiment itself was later begun (27°C for heat shocks, 27°C, 37°C or 47°C for growth experiments).

The cultures were inoculated to an initial OD_{620} of 0.2 for each growth experiment, and maintained continuously in shaking incubators (Multitron). Conversely, the cultures were inoculated to an initial OD of 0.05 for heat shock experiments. The culture was allowed to grow at 27°C up to an OD_{620} of 0.4 (approx. 1-1.5 x 10^8 cells per ml of culture) before performance of the heat shock to 47°C in a water bath with shaking function (Aquatron). Samples were then taken over a further two hours. The cell culture was then cooled in a second water bath for one hour to 27°C.

Determination of the glucose concentration in the medium

The glucose concentration in the medium was determined by means of the GOD method (GOD/POD Kit, Böhringer). Samples were diluted 1:200 with water. 190 µl 1% (w/v) GOD enzyme solution (supplied in powder form with the kit) was added to 10 µl of each sample and the mixture was incubated for approximately 25 minutes at 27°C. The glucose solution supplied in the kit was used as the standard, 10 µl (0.91 µg glucose) also being employed here. The absorption was measured in the Anthos 2001 spectrophotometer at 405 nm.

Extraction and quantitative detection f trehalose

Extraction of trehalose

1-10 ml of cell culture was filtered through a glass-fibre filter (Whatman GF/C) and washed three times with water. The filter was placed in an Eppendorf tube with 1 ml of water and vortexed for 30 seconds before being carefully squeezed out and removed. The cell suspension was then boiled for 10 minutes in the water bath. In order to separate the supernatant completely from the cell material, it was centrifuged three times at 20,000 g.

Determination of trehalose by HPLC

The extracted sugars were separated by means of a anion exchanger column (DIONEX CarboPac PA1 column, 4x250 mm) and detected amperometrically on a gold electrode (PED = pulsed electrochemical detector). The composition of the eluting gradient was as follows:

Time (minutes)	H_2O	H_2O	1 M Na acetate	1 M NaOH
0.0	45 %	45 %	0 %	10 %
3.5	40 %	39 %	0 %	21 %
4.5	35 %	35 %	20 %	10 %
5.0	45 %	45 %	0 %	10 %
14.0	45 %	45 %	0 %	10 %

These conditions resulted in a retention time for trehalose of approximately 3.7 minutes. 20 µl of sample was injected in each case. A 0.1 mg/ml trehalose solution was employed as the standard.

Determination of trehalose by enzymatic assay

An equally reliable enzymatic assay method was used in some cases as an alternative to the more expensive HPLC method (Parrou and François, 1997, modified): 25 µl of trehalose extract was mixed with 12.5 µl of trehalase (Sigma) and 37.5 µl buffer solution (0.2 M sodium acetate, 0.03 M $CaCl_2$, pH 5.7) and incubated for five hours at 37°C in a water bath. This resulted in complete breakdown of trehalose to two units of glucose. Following brief centrifugation, the samples were incubated for three minutes at 95°C and then centrifuged again for a further five minutes at 20,000 g. The trehalose concentration was determined indirectly by determination of the glucose concentration (GOD/POD kit, see above). 10 µl of this supernatant was used for this purpose.

Protein determination

Protein determination according to Peterson (slightly modified) (see Peterson, G.C. (1997) A simplification of the protein assay method of Lowry et al. which is more generally applicable. *Anal Biochem* **83**: 346-356)

In order to determine the total protein concentration of a cell culture 1 ml of cell suspension was precipitated in 1 ml 10% (w/v) TCA and centrifuged for 10 minutes at 3,000 g . The supernatant was drawn by means of a Pasteur pipette connected to a water-jet pump, and the sediment washed in 1 ml 1 N PCA. The pellet was then suspended in 5-12 ml (depending upon the OD of the cell culture to be studied) of a solution of 0.8 N NaOH:10% (w/v) SDS (1:1) and incubated for at least one hour at 60°C. 200 µl of this suspension has added thereto 600 µl 6x dilution of CTC reagent (10% Na_2CO_3, 0.1% $CuSO_4 \cdot 5H_2O$, 0.2% KNa tartrate). After exactly 10 minutes, 200 µl 6x dilution of Folin-Ciocalteu reagent was added and mixed briefly. The samples were left in the dark for 30 minutes, after which the absorption was measured at 750 nm, BSA serving as the standard.

Protein determination according to Bradford (1976)

In order to determine the protein concentration in cell-free extract, 100 µl of a suitably diluted extract was mixed with 700 µl of water. 200 µl of BioRad protein assay reagent

(Bradford) was then added and briefly shaken (Vortex). The absorption was measured at 595 nm, BSA serving as the standard.

Enzyme activity measurements

Preparation of permeabilized cells

The enzymatic activities of Tre6P synthase and neutral trehalase were measured in permeabilized cells (De Virgilio *et al.*, 1991). For this purpose, 1-6 ml of cells was filtered (on GF/C glass-fibre filters, Whatman), washed twice using ice-cold water, and resuspended by vortexing in 1 ml lyse buffer (0.2 M TRICIN, pH 7.0, 0.5% [v/v] Triton X-100). The filters were removed and the Eppendorf tubes frozen in liquid nitrogen and stored at -20°C. Prior to performance of the measurement, the cells were defrosted in a water bath for three minutes at 30°C. They were then washed twice in 0.2 M TRICIN (pH 7.0), and centrifuged for 20 s at 4°C and 8,000 rpm (Biofuge 17RS) after each wash. Finally, the cells were resuspended in 600 µl 0.2 M TRICIN (pH 7.0).

Tre6P synthase

The Tre6P synthase activity was determined by the coupled enzymatic assay according to Hottiger *et al.* (1987) at 50°C, 60 µl permeabilized cells always being employed. Both substrate (without Glu6P) and enzyme blanks (without permeabilized cells) were processed as controls.

Colony PCR with *H. polymorpha* cells

Colony PCR was performed according to a protocol by Huxley *et al.* (1990, modified): individual colonies were collected by means of a yellow pipette tip and scraped off in a PCR tube. The tubes were then heated for 2 minutes at full power in a microwave oven. Finally, 25 µl PCR mix (0.2 µl Taq polymerase, 2.5 µl 10x PCR buffer, 2.5 µl 25 mM $MgCl_2$, 0.5 µl 10 mM dNTP, 0.5 µM per final concentration of each primer and water added to bring the volume up to 25 µl) was added to each tube, and the cells

resuspended. The tubes were then immediately placed in the PCR unit, which was pre-heated to 92°C, and the program started.

Example 1: preparation of a radioactive TPS1 prob

Based upon a sequence comparison of the known *TPS1* genes of *S. cerevisiae, S. pombe, K. lactis, Candida albicans* and *A. niger* (see Figure 6), two degenerated primers could be prepared from two highly conserved regions; these amplified a fragment of approximately 650 bp during PCR (consisting of 30 cycles each comprising 1 minute at 92°C, 30 seconds at 52°C, 1 minute at 72°C) with genomic DNA from *H. polymorpha*. The sequences of the two primers were as follows:

F1 (forwards): 5' TGGCCVYTXTTCCAYTACCATCCYGG 3'
R1 (backwards): 5' GGCRTGBAAYTTYTGHGGHACACC 3'

B = C, G, T H = A, C, G R = A, G
V = A, C, G X = A, C, G, T Y = C, T

The PCR product was then loaded onto a preparative 1% (w/v) agarose gel and separated electrophoretically. The 650 bp band was cut out, extracted using the Geneclean II kit (Bio 101, Vista, USA), and labeled with radioactive [α-^{32}P]-dCTP. The Prime-It II kit was employed for this purpose, and the NucTrap columns for cleaning. This radioactive probe was used for the *TPS1* screen of *H. polymorpha* and for the Northern blot analysis.

Example 2: cloning of the TPS1 gene of *H. polymorpha*

Genomic DNA library of *H. polymorpha*:

The genomic DNA library used was made available by R. Hilbrands (University of Groningen, Netherlands). Preparation of the genomic DNA library is not critical, provided the fragments are ≥ approximately 2 kb. Genomic DNA fragments of *H. polymorpha* 2-5

kb in length (possibly several times this length) were cloned into the *BamHI* restriction site of pHRP2 (7813 bp). This plasmid (Faber *et al.,* 1992), K.N., Swaving, G.J., Faber, F., Ab, G., Harder, W., Veenhuis, M. and Haima, P. (1992) Chromosomal targeting of replicating plasmids in the yeast *Hansenula polymorpha. J Gen Microbiol* **138**: 2405-2416) contains an *ori* (replication origin) and an ampicillin-resistance gene for replication and selection in *E. coli.* The HARS1 sequence (*H. polymorpha* autonomously replicating sequence) and the *S. cerevisiae LEU2* gene acting as a marker, which also functions in *H. polymorpha* according to information provided by R. Hilbrands, are responsible for transformations in *H. polymorpha*. This library contains some 20,000 different clones.

Transformation of *E. coli*

The genomic DNA library was transformed by electroporation in *E. coli* (Sambrook *et al.*, 1989) and plated out onto 50 LB+Amp (75 mg/l) plates (2,000-4,000 colonies per plate). The plates were incubated overnight at 37°C.

Screening for the TPS1 gene of *H. polymorpha*

In order to permit analysis of the DNA of the individual colonies, nitrocellulose membranes were carefully placed on the plates (according to Sambrook *et al.*, 1989). A thin needle was used to produce four asymmetrically distributed holes through the membrane and gel. These acted as markers in order to enable the orientation of the membranes on the plates to be reproduced at a later stage. When the membranes were drawn, the colonies present on the plate were replicated.

Four plastic dishes containing 3MM absorbent paper (Whatman) were then laid out, and each dish moistened with one of four different solutions. Surplus liquid was discarded. The nitrocellulose membranes were first placed (with the colonies facing upwards) on absorbent paper soaked in 10% (w/v) SDS for 3 minutes. They were then placed in the second dish containing denaturing solution (0.5 N NaOH, 1.5 M NaCl) for 5 minutes. Then they were held one after the other on absorbent paper with neutralizing solution (1.5 M NaCl, 0.5M Tris-HCl, pH 7.4) and with 2x SSC (10x SSC 1.5 M NaCl, 170 mM sodium citrate), for 5 minutes each. In order to fix the DNA to the nitrocellulose, each

membrane was placed between two 3MM absorbent papers and baked in a vacuum oven at 80°C for 2 hours. The membranes were then moistened for 5 minutes in 2x SSC, before being dipped for 30 minutes in a prewash solution at 50°C (5x SSC, 0.5% [w/v] SDS, 1 mM EDTA, pH 8.0). A wet Kleenex was used to wipe away surplus bacterial material before the membranes were placed for 2 hours in pre-hybridization solution (6x SSC, 0.25% [w/v] skim-milk powder) at 68°C. For the main hybridization process, approximately 1 x 10^7 cpm of radioactively labeled *TPS1* probe (refer to "preparation of a radioactive *TPS1* probe") was placed in 40 ml pre-hybridization solution, and the membranes incubated in it overnight at 68°C. Following brief rinsing of the membranes three times in 2x SSC, 0.1% (w/v) SDS and washing for 1 hour at 68°C in 1x SSC, 0.1% (w/v) SDS, the membranes were dried and exposed on BioMax film. The signals on the developed films enabled 8 positive colonies to be picked on the plates and stocks to be created from them. The plasmids were extracted from these colonies. PCR was employed to test whether the 650 bp fragment was in fact present.

Example 3: sequencing of the *TPS1* gene of *H. polymorpha*

Plasmid isolation

For sequencing, two colonies were selected which, by means of PCR with primers from within the 650 bp fragment outwards (F4 and R4, see Table 1) and from the plasmid towards the insert (Plasm. F and Plasm. R, see Table 1) yielded the largest possible bands. Pure plasmid extracts were prepared from these two colonies (Nos. 20.1 and 21.3) by means of the Plasmid Midi Kit (Qiagen).

Sequencing

Sequences were produced by means of a cyclical sequencing program (PCR apparatus: Progene) and the ABI 301 automatic sequencer (Perkin Elmer). 0.5 µl (0.5 µg) plasmid DNA, 1 µl primer (final concentration 0.5 µM), 4 µl reaction mixture (DNA sequencing kit) and 4 µl water were used for this purpose. The sequencing program employed involved 27 cycles comprising 30 seconds at 96°C, 15 seconds at 50°C, and 4 minutes at 60°C. Upon completion of the program, 10 µl water was added to the reaction, and the DNA

precipitated with sodium acetate and ethanol. The pellet was washed twice using 1 ml ice-cold 70% (v/v) ethanol. The DNA was then dried briefly and resuspended in 25 µl TSR (template suppressing reagent, DNA Sequencing Kit). Following incubation for two minutes, the samples were then ready for sequencing in the ABI 301.

The primers employed for sequencing the plasmid No. 21.3 (see Fig. 2.1) are listed in Table 1. They were prepared at the FMI on "Expedite™ Nucleic Acid Synthesis" equipment. The sequences were analyzed by means of the GCG program (Devereux *et al.*, 1984).

Table 1: List of primers employed for sequencing the *TPS1* gene

Name	Direction	Length (bp)	Sequence
F3	Forwards	23	5' GGAAGCAAATAAACTGTTTTGCC 3'
F4	Forwards	23	5' CTGTAAGTGCTTATCCGATTGGC 3'
F6	Forwards	22	5' GGACGACAAACTGTCGAGCGGG 3'
F7	Forwards	22	5' CATACTCCTTTTCCTTCAAGCG 3'
F8	Forwards	21	5' AAAGCGTGAACTTCCAAGAGC 3'
F9	Forwards	22	5' GCGTGTGATTACTGTGGTTTGC 3'
F10	Forwards	26	5' GGTGAGATAATATTTTCGAAATTTCC 3'
F11	Forwards	27	5'CCCATCAAATGCAGCAAGATATTGACC3'
R3	Backwards	21	5' CCATTCAAGAATTTGTCAACG 3'
R4	Backwards	23	5' CATGAGATGATAATCATGTACCC 3'
R5	Backwards	23	5' CAATTTTGACATTCGGTAGCCCC 3'
R6	Backwards	22	5' GTAATGCCGTCACTAATCCGCC 3'
R7	Backwards	23	5' GAACATCTTCTGAAAATTGCCCC 3'
R8	Backwards	21	5' CTAGCTCATTTACAGCTGCCC 3'

Name	Direction	Length (bp)	Sequence
R9	Backwards	25	5' CATAGCTTTCGAGCCTTTCATCTGG 3'
Plasm F	Forwards	24	5' GGCGAGCCCGATCTTCCCCATCGG 3'
Plasm R	Backwards	26	5' CTGCTCGCTTCGCTACTTGGAGCCAC3'

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Claims

1. Heat-inducible promoter, characterized in that it contains or consists of a partial sequence which exhibits at least 40% identity over a length of 300 bp with the following sequence or the complementary sequence:

```
-792  CTTAAATACCACAATAGGAAAATTATCAATAAAGCTTTTCGGATTTCATTACGTTATATC -733
-732  GCAAAAAAATAGTCGAGCTTTCTGAACCGTTCGTTAATAAAAAAATAGTTTTTTCAGATT -673
-672  TCTATGTGAGGCAGTCACGATAGAATTCCATCGAACTCGTCAGCGCCAAATGTGAATGCG -613
-612  GCTTTCAAAAGCTTTGTCGAATTTGGGATGGGAATCCATGAATCGAAGATGTCAAAATGG -553
-552  GGGATCACAAAAGTACACTCACGAGGAAAATCAAAACCTTCTCGTACCTTTAACACATAC -493
-492  GGAAATGATCGATCGATTTGAGAAGATTCCTCAATGATTTTCGTCATATATAGGTATCTG -433
-432  AGGTATTTATGGACCGATTCGTAATAACATCATATACATCGCGCTTTGTCCCTGTCCCAG -373
-372  AGATTTCGATGAAAAAAGCGAATTTTATTCTAATATTTGAAGCATGCCAAACATGGGGCA -313
-312  GTTGATTTGTGTGAGGGTAAAATATCATGAATTGCACCCATCAAATGCAGCAAGATATTG -253
-252  ACCAATCCTATAATAGAAAACAGACTTACCACAAATAGATTGTGATGACGATATTATGAA -193
-192  TCTCCAGATGAAAGGCTCGAAAGCTATGAAGCCTCTTGAAACTTTTCATGGTGAGATAAT -133
-132  ATTTTCGAAATTTCCACGAACTTCTAAAACGCAATTATTGAATATAAAGGAAAAATAATA -73
 -72  TTTCCATATAGCAAGCAAATCAAGCTGCACTCCTCATCCTTAAAACTAATAAATCTTACC -13
 -12  CATTTGATACCA
```

2. Promoter according to claim 1, characterized in that within the partial sequence it contains no STRE element having the sequence CCCCT or AGGGG.

3. Promoter according to claim 1 or 2, characterized in that within the partial sequence it exhibits at least one heat shock element with the sequence NGAANNXYZNNGAAN or the complementary sequence thereof, where N, X, Y and Z, independently of one another, may be A, T, C and G, where X is preferably G, C or T, Y preferably A or T, and Z preferably C or A.

4. Promoter according to any one of the preceding claims, characterized in that the heat shock elements are selected from TGAAGCCTCTTGAAA and/or TGAATATAAAGGAAA and/or the complementary sequences thereof, wherein the same or different sequences may be present.

5. Promoter according to any one of the preceding claims, characterized in that it exhibits at least two different heat shock elements.

6. Promoter according to any one of the preceding claims, characterized in that it exhibits at least the sequence from –166 to –80.

7. Promoter according to any one of the preceding claims, characterized in that it contains or consists of the sequence from –300 to –1.

8. Promoter, in particular according to any one of the preceding claims, characterized in that it comprises a promoter sequence of the promoter of the *TPS1* gene which is obtainable through isolation from a genomic library of *Hansenula polymorpha*.

9. Promoter according to any one of the preceding claims, characterized in that it contains the partial sequence several times.

10. Method for isolating a promoter according to any one of claims 1 to 9, characterized in that a probe is produced with the coding sequence of *TPS1* as the "template" and that a DNA library, in particular a genomic DNA library, is screened therewith, the probe being preferably produced in a length of at least 200 – 400 bp and by using primers which preferably have a length of 20 – 21 bp.

11. Use of a promoter according to any one of claims 1 to 9 for the expression of a foreign gene in fungi, in particular in yeasts, specifically preferably in *Hansenula polymorpha*.

12. Amino acid sequence, characterized in that it exhibits at least 80% identity with the sequence of *TPS1* of *Hansenula polymorpha* indicated in Table 1, preferably 90%, specifically preferably 100% identity.

13. Amino acid sequence, characterized in that it is at least one partial sequence of the sequence defined in claim 12 and exhibits trehalose-6-phosphate synthase activity.

14. DNA sequence coding for the amino acid sequence according to claim 12 or 13.

15. DNA sequence according to claim 14, characterized in that it codes with the sequence illustrated in Table 1 or with a sequence which in consideration of the degeneration of the genetic code encodes a corresponding amino acid sequence, exhibits at least 80%, preferably 90%, particularly preferably 100%, identity or complementarity or that it hybridizes under stringent conditions with the sequence shown in Table 1 or with a sequence which in consideration of the degeneration of the genetic encodes a corresponding amino acid sequence.

Abstract

A selectively heat-induced promoter, its preparation and its use, as well as means for the preparation thereof, are described. The promoters concerned are promoters derived from the promoter of the trehalose-6-phosphate synthase gene of *H. polymorpha*. They are suited as promoters for the expression of foreign genes in fungi, specifically yeasts, in particular *H. polymorpha*.

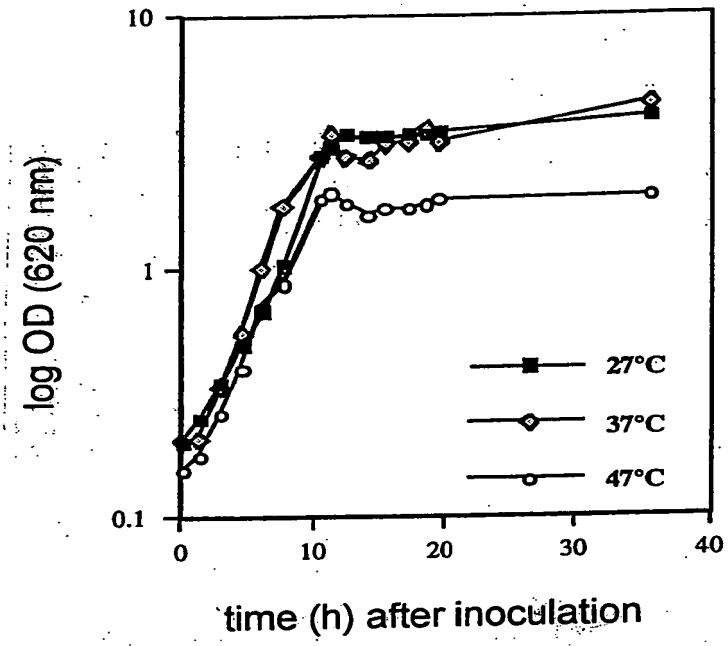


Figure 1

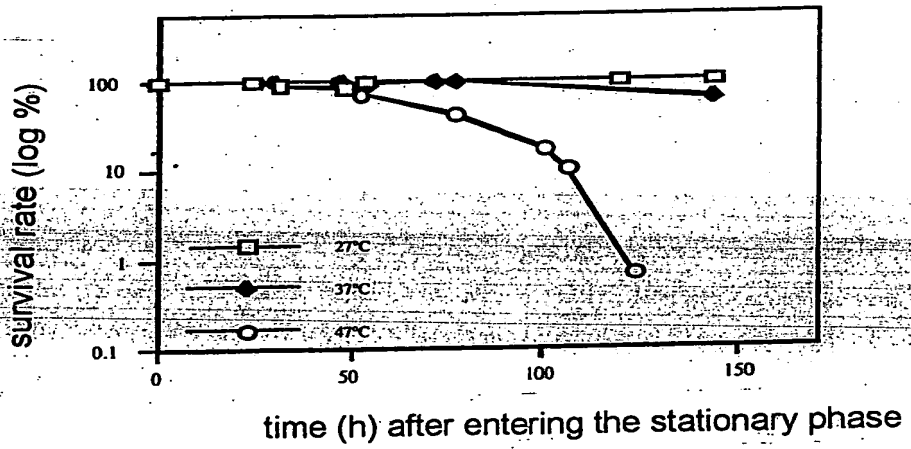
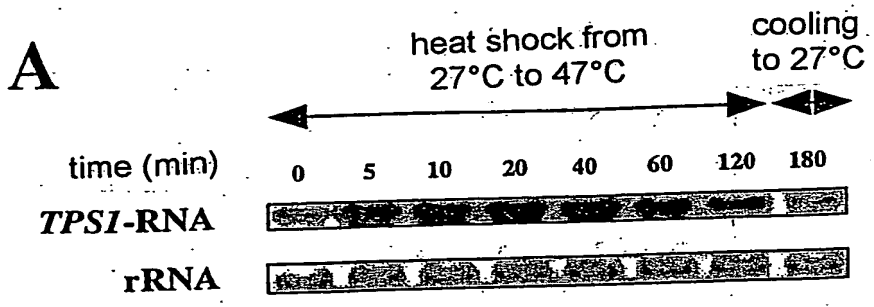
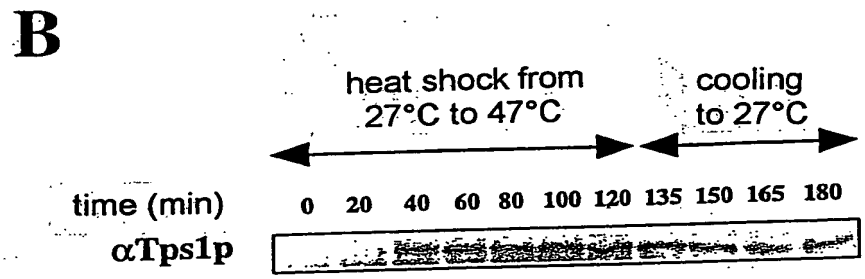


Figure 2





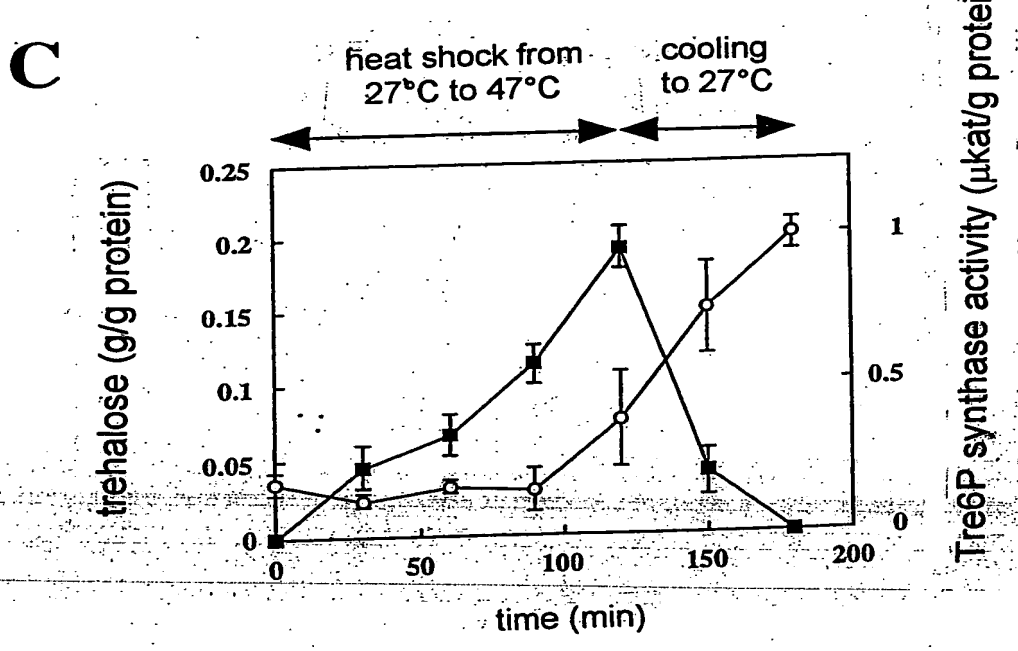


Figure 3

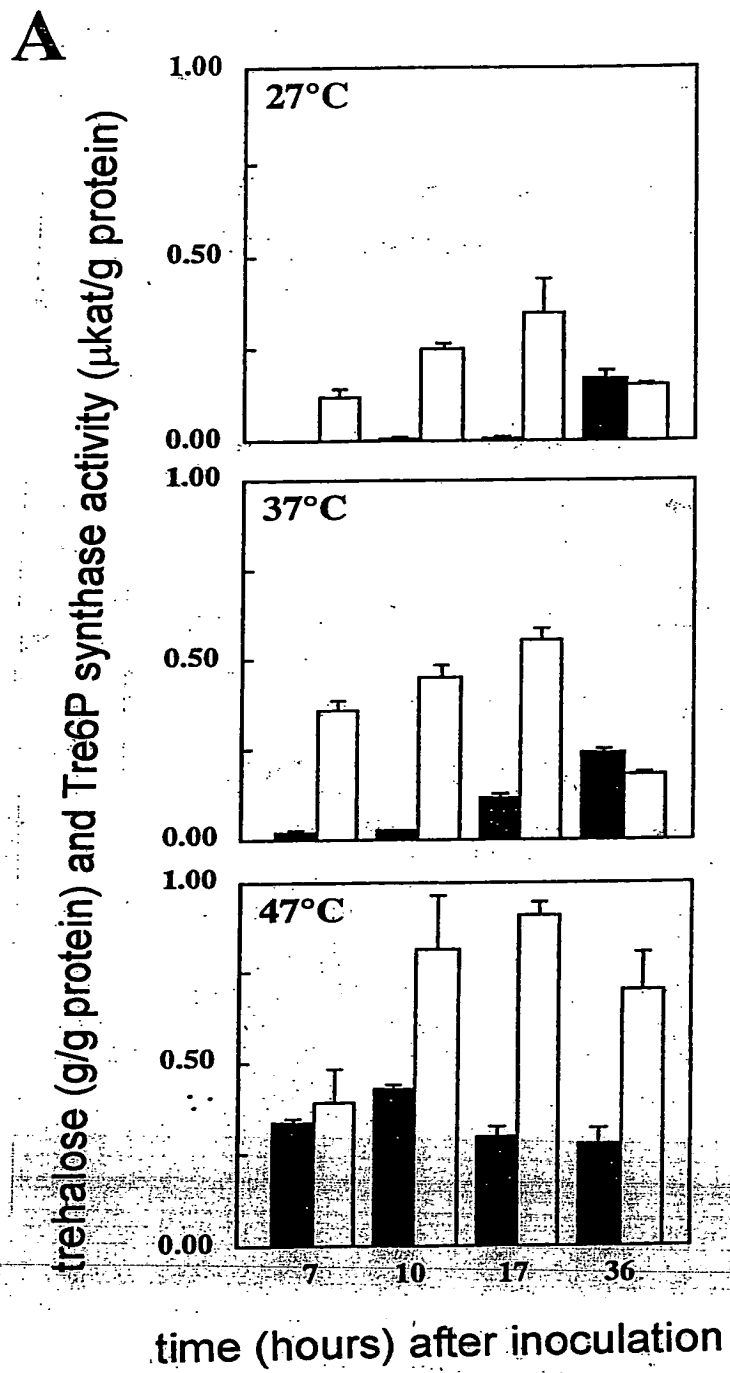
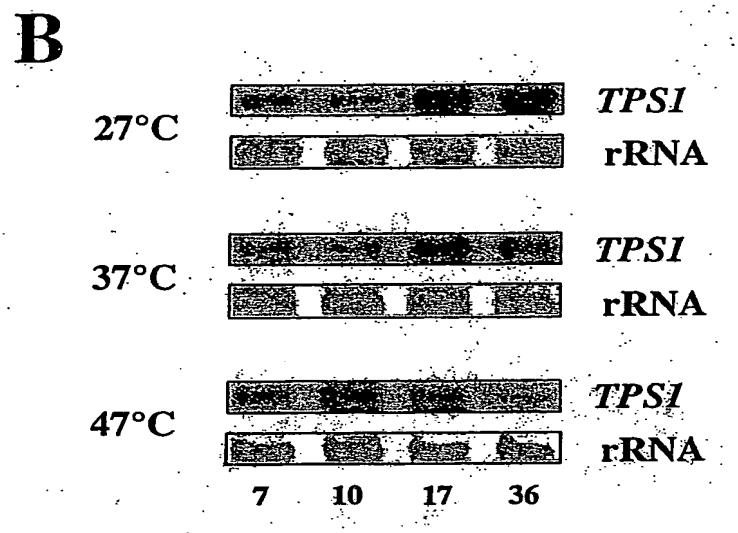


Figure 4 A



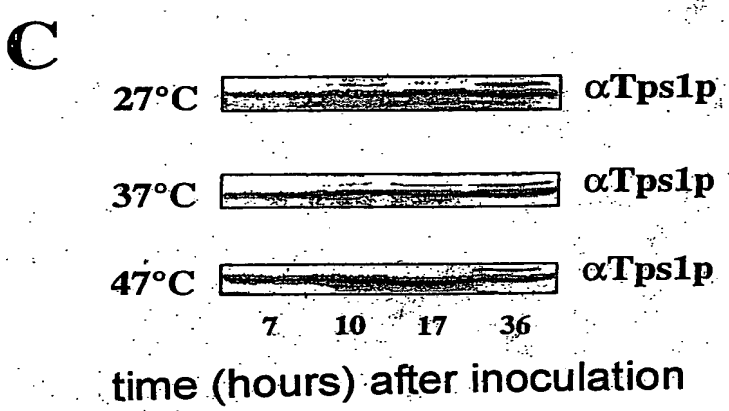


Figure 4 B, C

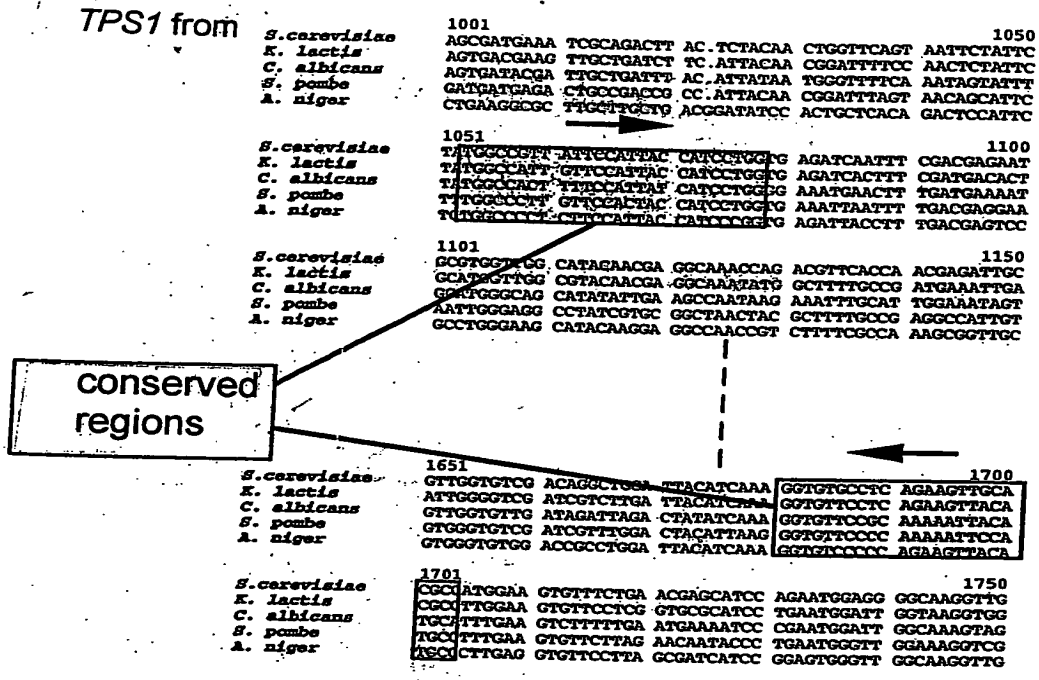


Figure 5